



UNIVERSITI PUTRA MALAYSIA

**POSSIBLE ANTINOCICEPTIVE MECHANISM AND SITE OF ACTIVITY
OF HARUAN (*Channa striatus*) CRUDE AQUEOUS EXTRACT IN MICE**

ZAINUL AMIRUDDIN B. ZAKAFUA

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By

ZAINUL AMIRUDDIN B. ZAKARIA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

October 2005



DEDICATION

This thesis is dedicated to all of the following people who have inspired me in a very special way that only I can understand:

MYSELF ~ For what I am!!!

MAK and ABAH ~ For bringing me here!!!

SHARIAH LOH LONG ~ For her eternal love and patience!!!

MUHAMMAD AFIQ AHLAMI ~ For reminding me not to give up!!!

MY FAMILY ~ For being there!!!

MY SUPERVISOR and CO-SUPERVISORS ~ For trusting me!!!

“It is better to burn out than to fade away...

***Peace. Love. Empathy.*”**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

POSSIBLE ANTINOCICEPTIVE MECHANISM AND SITE OF ACTIVITY OF HARUAN (*Channa striatus*) CRUDE AQUEOUS EXTRACT IN MICE

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Chairman: Associate Professor Mohd. Roslan Sulaiman, PhD

Faculty : Medicine and Health Science

The present study was carried out to determine the possible mechanism of antinociception and site of activity of the crude aqueous extract of Haruan (*Channa striatus*) (ASH) in mice using the abdominal constriction test. The ASH, obtained after chloroform:methanol (CM) (2:1; v/v) extraction (24 hrs) of the fresh Haruan fillet, was evaporated to remove the methanol residue and used throughout the study. The first study was carried out to ascertain the dry weight and antinociceptive profile of ASH. The second study was carried out to determine the amino acids and fatty acids compositions, as well as the polypeptide profile of ASH. The third study was carried out to determine the actual onset and offset of ASH activity after its subcutaneous (SC) or intraperitoneal (IP) administration at four different sets of time (0, 5, 30 and 60 min). The fourth and fifth studies were carried out to determine the involvement of opioid and non-opioid receptors, respectively, in the ASH antinociceptive activity. All of the antagonists of opioidergic, muscarinic, nicotinic, α - and β -adrenergic, dopaminergic, serotonergic and γ -aminobutyric acid (GABA) receptors were administered (SC) 10 min prior to ASH (SC) administration. The sixth study was carried out to determine the role of L-

arginine/nitric oxide/cyclic 3':5'-guanosine monophosphate (L-arginine/NO/cGMP) pathway in the ASH antinociceptive activity. The precursor (L-arginine) and inhibitor (N^G -nitro-L-arginine methyl esters (L-NAME)) for NO, as well as the inhibitor for cGMP (methylene blue (MB)), were administered (SC) 5 min before ASH administration (SC). In all of the above-mentioned studies that involved the use of antinociceptive test, the 0.6% acetic acid-induced abdominal constriction test in mice was used as an assay to evaluate the ASH antinociceptive activity. All data obtained were analysed using the One-way Analysis of Variance (ANOVA) followed by the Tukey test with $P < 0.05$ as the limit of significance.

From the data obtained, the ASH, which exhibited significant ($P < 0.05$) and concentration/dosage-dependent antinociceptive activity, yielded 1.89g/10.0ml of white coloured powder after subjection to the freeze-drying process. The ASH was also found to contain all the important amino acids with major amino acids found are glycine ($35.77\% \pm 0.58$), alanine ($10.19\% \pm 1.27$), lysine (9.44 ± 0.56), aspartic acid (8.53 ± 1.15) and proline ($6.86\% \pm 0.78$). Furthermore, the ASH was also found to contain high composition of palmitic acid (C16:0) ($35.93\% \pm 0.63$), oleic acid (C18:1) ($22.96\% \pm 0.40$), stearic acid (C18:0) ($15.31\% \pm 0.33$), linoleic acid (C18:2) ($11.45\% \pm 0.31$) and arachidonic acid (C20:4) ($7.44\% \pm 0.83$). The ASH was also found to produce at least four major fractions (at the retention times of 8.919, 9.841, 10.263 and 10.744), when subjected to the high performance liquid chromatography (HPLC) process, that are believed to be polypeptides. The onset time and the offset time of the ASH antinociceptive activity, which are concentration-dependent and concentration-

independent, occurred between 0 to 5 min, and 60 min after its SC administration. Interestingly, changing the route of administration from SC to IP caused significant ($P<0.05$) increase in the ASH antinociceptive activity with the concentration-independent onset time of activity observed immediately after the ASH administration with no apparent offset time. The activity was found to reach the maximum effect 30 min after the ASH administration regardless of the route of administration used. Pretreatment with naloxone at all dosages did not cause any significant changes in the ASH antinociceptive activity indicating that the activity did not involve an opioid receptor mechanism, and thus confirmed the report made by Dambisya *et al.* (1999). Pre-treatment with various types of non-opioid receptor antagonists demonstrated the involvement of at least four types of receptors (muscarinic, GABA_A, α -adrenergic and serotonergic) in the mechanism of ASH antinociceptive activity. Pre-treatment with atropine and bicuculine almost completely blocked ($P<0.05$), while pre-treatment with phenoxybenzamine and methysergide significantly ($P<0.05$) reduced half of the ASH activity. The role of L-arginine/NO/cGMP pathway in ASH antinociceptive activity was also observed after pretreatment of the ASH with L-arginine, L-NAME or MB, but not with D-arginine. Pretreatment with L-arginine was found to significantly ($P<0.05$) reduce the ASH antinociceptive activity, whereas pretreatment with L-NAME or MB were found to enhance ($P<0.05$) the activity. Based on the finding, low concentration of NO, limited by the presence of higher concentration of ASH, and inhibition of cGMP system play important role in ASH antinociceptive activity. However, the actual mechanism underlying this phenomenon is yet to be fully understood.

As a conclusion, we suggest that the ASH-produced antinociceptive activity could be due to the presence of various types of amino acids and fatty acids, as well as four major fractions, and involved activation of at least four types of the non-opioid receptors (namely the muscarinic, GABA_A, α -adrenergic and serotonergic) and the L-arginine/NO/cGMP pathway.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan ijazah Doktor Falsafah

**MEKANISMA DAN TAPAK TINDAKAN ANTINOSISEPTIF YANG MUNGKIN
BAGI EKSTRAK AKUES KASAR HARUAN (*Channa striatus*) DALAM MENCIT**

Oleh

ZAINUL AMIRUDDIN B. ZAKARIA

Oktober 2005

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Kajian terkini ini dijalankan untuk menentukan mekanisme dan tapak tindakan antinosiseptif yang mungkin terlibat bagi ekstrak akues kasar Haruan (*Channa striatus*) (ASH) dengan menggunakan ujian pencerutan abdominal keatas mencit. ASH, diperolehi selepas pengekstrakan (24 jam) filet segar Haruan menggunakan kloroform:metanol (CM) (2:1; v/v), dievaporasikan untuk menyingkirkan residu metanol dan digunakan sepanjang kajian dijalankan. Kajian pertama dijalankan bagi menentukan berat kering dan profil antinosiseptif bagi ASH. Kajian kedua dijalankan untuk menentukan komposisi asid-asid amino dan asid-asid lemak, serta profil polipeptida bagi ASH. Kajian ketiga dijalankan untuk menentukan masa sebenar bermula dan berakhirnya aktiviti antinosiseptif ASH selepas pemberian secara subkutaneus (SC) atau intraperitoneal (IP) pada empat set masa yang berbeza (0, 5, 30 dan 60 min). Kajian keempat dan kelima dijalankan untuk menentukan penglibatan reseptor-reseptor opioid dan bukan-opioid, masing-masing, dalam aktiviti antinosiseptif ASH. Kesemua antagonis-antagonis bagi reseptor-reseptor opioidergik, muskarinik, nikotinik, α - dan β -adrenergik, dopaminergik, serotoninergik dan asid γ -aminobutirik (GABA), diberikan (SC) 10 min sebelum

pemberian ASH (SC). Kajian keenam dijalankan untuk menentukan peranan laluan L-arginina/nitrik oksida/ 3':5'- guanosina monofosfat siklik (L-arginine/NO/cGMP) dalam aktiviti antinosiseptif ASH. Prekursor (L-arginina) dan perencat (N^G -nitro-L-arginina metil ester (L-NAME)) bagi NO, serta perencat (metilena biru (MB)) bagi cGMP, diberikan (SC) 5 min sebelum pemberian ASH (SC). Dalam kesemua kajian yang dinyatakan di atas yang melibatkan penggunaan ujian antinosiseptif, ujian pencerutan abdominal keatas mencit yang dicetus oleh 0.6% asid asetik telah digunakan sebagai assai untuk menilai aktiviti antinosiseptif ASH. Kesemua data yang telah diperolehi dianalisa menggunakan *One-way Analysis of Variance (ANOVA)* diikuti oleh ujian *Tukey* dengan $P < 0.05$ sebagai had bererti.

Dari data yang telah diperolehi, ASH, yang menunjukkan aktiviti antinosiseptif yang bererti ($P < 0.05$) dan bergantung kepada kepekatan/dos, telah menghasilkan 1.89g/10.0ml serbuk berwarna putih selepas menjalani proses beku-kering. ASH juga didapati mengandungi kesemua asid-asid amino penting dengan asid-asid amino utama yang didapati adalah glisina ($35.77\% \pm 0.58$), alanina ($10.19\% \pm 1.27$), lisina (9.44 ± 0.56), asid aspartik (8.53 ± 1.15) dan prolina ($6.86\% \pm 0.78$). Tambahan pula, ASH juga didapati mengandungi komposisi tinggi asid palmitik (C16:0) ($35.93\% \pm 0.63$), asid oleik (C18:1) ($22.96\% \pm 0.40$), asid stearik (C18:0) ($15.31\% \pm 0.33$), asid linoleik (C18:2) ($11.45\% \pm 0.31$) dan asid arakidonik (C20:4) (7.44 ± 0.83). ASH juga didapati menghasilkan empat fraksi utama (pada masa penahanan iaitu 8.919, 9.841, 10.263 dan 10.744) apabila didedahkan kepada kromatografi cecair berprestasi tinggi (HPLC), yang dipercayai adalah polipeptida-polipeptida. Masa bermula dan berakhirnya aktiviti

antinosiseptif ASH, yang bergantung dan tidak bergantung kepada kepekatan, masing-masing terhasil diantara 0 dan 5 min, dan 60 min selepas pemberiannya secara SC. Yang menariknya, penukaran kaedah pemberian dari SC kepada IP menyebabkan peningkatan yang bererti ($P < 0.05$) dalam aktiviti antinosiseptif ASH dengan masa bermulanya aktiviti, yang tidak bergantung kepada kepekatan, dilihat serta-merta selepas pemberian ASH tanpa masa berakhir yang nyata. Aktiviti tersebut juga didapati mencapai kesan maksimum 30 min selepas pemberian ASH tanpa mengambilkira kaedah pemberian yang digunakan. Pra-rawatan dengan nalokson pada semua dos tidak menyebabkan sebarang perubahan bererti dalam aktiviti antinosiseptif ASH menunjukkan bahawa aktiviti tersebut tidak melibatkan mekanisme reseptor opioid dan, dengan itu mengesahkan laporan yang dibuat oleh Dambisya *et al.* (1999). Pra-rawatan dengan pelbagai jenis antagonis-antagonis reseptor menunjukkan penglibatan sekurang-kurangnya empat jenis reseptor (muskarinik, GABA_A, α -adrenergik and serotonergik) dalam mekanisme aktiviti antinosiseptif ASH. Pra-rawatan dengan atropina dan bikukulina menghalang hampir keseluruhan ($P < 0.05$), manakala pra-rawatan dengan fenoksibenzamina dan methysergide menurunkan secara bererti ($P < 0.05$) separuh dari aktiviti ASH. Peranan laluan L-arginine/NO/cGMP dalam aktiviti antinosiseptif ASH juga dapat dilihat selepas pra-rawatan keatas ASH dengan L-arginina, L-NAME atau MB, tetapi tidak D-arginina. Pra-rawatan dengan L-arginine telah didapati menurunkan aktiviti antinosiseptif ASH secara bererti ($P < 0.05$), manakala pra-rawatan dengan L-NAME atau MB telah didapati meningkatkan ($P < 0.05$) aktiviti itu. Berdasarkan penemuan ini, kepekatan rendah NO, yang mana dipengaruhi oleh kehadiran ASH dalam kepekatan tinggi, dan perencatan sistem cGMP memainkan peranan penting dalam aktiviti antinosiseptif ASH.

Walaupun bagaimanapun, mekanisme sebenar yang mendasari fenomena ini masih belum dapat difahami sepenuhnya.

Sebagai kesimpulannya, kami mencadangkan bahawa aktiviti antinosiseptif yang dihasilkan oleh ASH adalah disebabkan oleh kehadiran pelbagai jenis asid-asid amino dan asid-asid lemak, serta kehadiran sekurang-kurangnya empat jenis fraksi-fraksi utama, dan melibatkan pengaktifan, sekurang-kurangnya, empat jenis reseptor-reseptor bukan opioid (iaitu muskarinik, GABA, α -adrenergik dan serotonergik) dan laluan L-arginine/NO/cGMP.

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All gratifications are referred to ALLAH S.W.T.”

I would like to take this opportunity to thank the chairman of my supervisory committee, Associate Professor Dr. Mohd. Roslan B. Sulaiman, who has been like a brother to me, whose expert guidance and support has helped me to complete this research. His kindness, affection, encouragement and moral support gave me the courage and ability to overcome all the problems I have faced from time to time during the course of my work. I would like to extend my heartfelt appreciation to him for his invaluable advice and continuous comments, which brighten my future through the experiences that I have gained from him.

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I certify that an Examination Committee met on October 3rd 2005 to conduct the final examination of Zainul Amiruddin B. Zakaria on his Doctor of Philosophy thesis entitled "Possible Antinociceptive Mechanism and Site of Activity of Haruan (*Channa striatus*) Crude Aqueous Extract in Mice" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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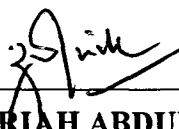
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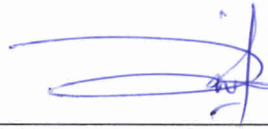


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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



ZAINUL AMIRUDDIN B. ZAKARIA

Date: 23/12/2005

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